The Virus BioResistor: Wiring Virus Particles for the Direct, Label-Free Detection of Target Proteins

Apurva Bhasin[1†], Alana F. Ogata[1†], Jeffrey S. Briggs[2], Phillip Y. Tam[2], Ming X. Tan[3], Gregory A. Weiss[1,2*], Reginald M. Penner[1,2*]

[1]*Department of Chemistry, University of California, Irvine, Irvine, CA 92697-2025,*
[2]*PhageTech Inc., 5151 California Ave. Suite 150, Irvine, CA 92617,*
[3]*Wainamics Inc., 3135 Osgood Ct, Fremont, CA 94539.*

Supporting Information

EXPERIMENTAL METHODS.

Materials. Devices were designed and manufactured by the Penner lab. Devices and cells (Wainamics Inc., Fremont CA) were cleaned by O_2 plasma using a plasma cleaner (PDC-32G, Harrick Plasma) All chemicals were purchased from Sigma Aldrich and used as received, unless noted. Buffer solutions contained phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 8) filtered through a 0.22 μM pore size membrane (Corning) and 2 mg/mL of Casein. Human serum albumin (Human Albumin fraction V; low folate, B12; MP Biomedicals; purity > 97%) were analyzed by SDS-PAGE and used as received. Bovine serum albumin (BSA, Calbiochem Omnipur) was used as received. EDOT was purified by silica flash chromatography.

Phage Library Design and the Selection of HSA Binders. The procedures associated with design of the phage library, selection of HSA binders from this library, and screens to isolate the HSA-binding virus were described previously (ref. 17).

VBR Fabrication. The fabrication process for one pair (2) of *VBR*s involved the following four steps (Figure S1). First, two pairs of gold-electrodes are prepared by photolithography. These gold electrodes have width of 2 mm and their separation of 1.5 mm defines the *VBR* channel length. Gold electrode pairs are separated laterally by 0.5 mm. Second, a layer of PEDOT:PSS is spin-coated onto the gold-electrode device and baked for 1 hr at 90˚C. Third, a 2 mm x 2 mm PMMA cell is attached defining the area of the *VBR* bioaffinity layer. Fourth and finally, a virus-PEDOT top layer is electropolymerized on top of the PEDOT-PSS bottom layer by using ≈100 μL of plating solution and applying two oxidizing voltammetric scans. The virus-PEDOT plating solution is removed and the cell is rinsed. Electrodes are used to enable impedance measurements at each of the two VBR sensors. One background impedance measurement is acquired in buffer, and a second in a solution containing added HSA. The difference between these two measurements, calculated at each frequency, is ΔR_{VBR}.

Further details relating to this process are the following: Gold-film electrodes were cleaned by O_2 plasma for 10 min immediately before use. Scotch tape was placed on the ends of the

electrodes to protect the contacts. A poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS,1.0 wt.% in H_2O, high conductivity grade) layer was deposited on the electrodes by spin-coating (2500 rpm, 80s) and baked for 1 hr at 90˚ C. Electrodes were then allowed to come to room temperature and the cell was then mounted on the gold-film electrodes followed by the incubation of the electrodes in PBS for 90 min. Next, virus-PEDOT films were electropolymerized onto the PEDOT:PSS/gold-film electrodes using a platinum foil counter and a mercurous sulfate electrode (MSE). Virus-PEDOT films were prepared by cycling between 0.2 V and 0.8 V at a scan rate of 20 mV/s in plating solution using a PARSTAT 2263 controlled by Electrochemistry PowerSuit 2.6 software. Plating solutions contained 8 nM M13 bacteriophage, 12.5 mM $LiClO_4$, 2.5 mM EDOT and electropolymerized for 2 cycles.

*VBR*s were evaluated at every step of the fabrication process to ensure the reproducibility of signal for a particular HSA concentration. Starting with the fabrication by photolithography of gold electrodes, the *VBR* is prepared in four steps (Figure S1). The parameters measured at each of these steps is indicated in the diagram for Fig. S1. In this diagram, the following definitions apply: $\boldsymbol{R_{au}}$ is the dc resistance of the gold electrons prepared in step 1, measured along their longest dimension, $\boldsymbol{R_{trans}}$ is the dc resistance of the PEDOT-PSS film produced in step 2, $\boldsymbol{i_p}$ is the peak current for the electropolymerization, by cyclic voltammetry, of the virus-PEDOT composite in step 3, $\boldsymbol{Z_{im,m}}$ and $\boldsymbol{Z_{re,m}}$ are the baseline impedances measured for the complete *VBR* measured in 160 mM PBS buffer solution. *VBR* device yield using these process windows was ≈30%.

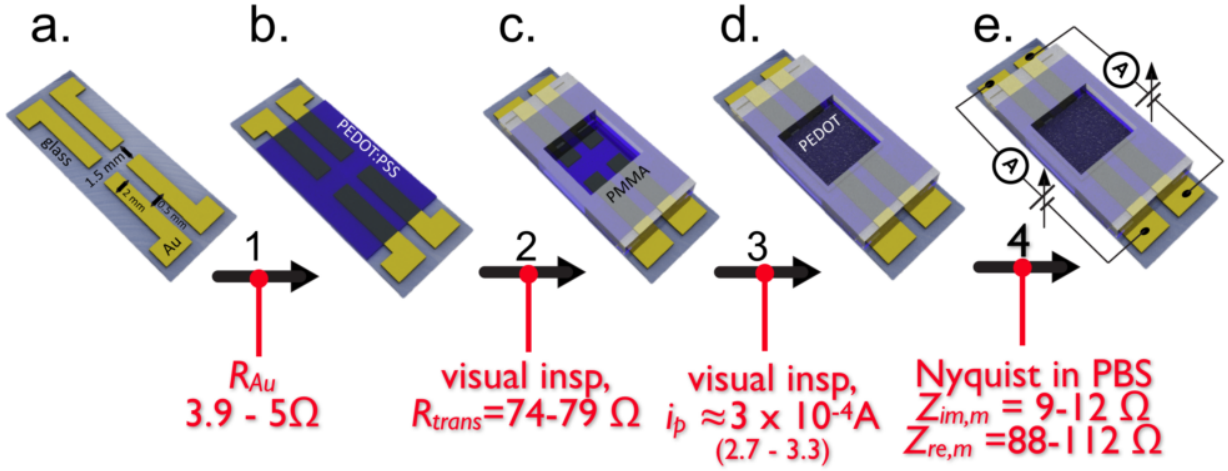

Figure S1 – Process flow for the four-step *VBR* fabrication process, including the process windows that were enforced for this process, indicated in red.

Impedance Spectroscopy (IS). All buffer solutions were prepared and brought to room temperature (74˚F) prior to EIS measurements. Newly plated phage-PEDOT chemiresistor films were washed three times with PBS and subsequently incubated in PBS for 10 minutes. All EIS measurements were taken with the PBS solution as the run buffer. Three consecutive IS measurements were taken using a PARSTAT 2263 controlled by Electrochemistry PowerSuit 2.6 software. 50 data points were acquired across a frequency range of 5 Hz to 40 kHz. The amplitude of the applied voltage was 10 mV for all EIS measurements. Chemiresistor films were then incubated in HSA solutions in run buffer for 10 min followed by three consecutive IS measurements. Independent electrodes were used for measurements of a single concentration of HSA solutions and BSA solutions. Equivalent circuit fitting was acquired using the EIS Analyzer software (ABC Chemistry).

AFM and SEM Analysis. Scanning electron microscopy (SEM) were acquired on uncoated films at 2 keV using a FEI Magellan 400L XHR system. Atomic force microscopy (AFM) was performed on chemiresistor films using an Asylum MFP-3D-SA atomic force microscopy (Asylum Research, Santa Barbara, CA) equipped with Olympus AC160TSAFM tips (Olympus) in laboratory ambient air. AC Mode AFM images were acquired at 512 x 512 pixels spanning a 20 μm range. The Asylum image processing software was used to analyze AFM images and amplitude traces.